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Urbanization reduces genetic connectivity in bobcats (*Lynx rufus*) at both intra- and interpopulation spatial scales

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Abstract

Urbanization is a major factor driving habitat fragmentation and connectivity loss in wildlife. However, the impacts of urbanization on connectivity can vary among species and even populations due to differences in local landscape characteristics, and our ability to detect these relationships may depend on the spatial scale at which they are measured. Bobcats (*Lynx rufus*) are relatively sensitive to urbanization and the status of bobcat populations is an important indicator of connectivity in urban coastal southern California. We genotyped 271 bobcats at 13,520 SNP loci to conduct a replicated landscape resistance analysis in five genetically distinct populations. We tested urban and natural factors potentially influencing individual connectivity in each population separately, as well as study-wide. Overall, landscape genomic effects were most frequently detected at the study-wide spatial scale, with urban land cover (measured as impervious surface) having negative effects and topographic roughness having positive effects on gene flow. The negative effect of urban land cover on connectivity was also evident when populations were analyzed separately despite varying substantially in spatial area and the proportion of urban development, confirming a pervasive impact of urbanization largely independent of spatial scale. The effect of urban development was strongest in one population where stream habitat had been lost to development, suggesting that riparian corridors may help mitigate reduced connectivity in urbanizing areas. Our results demonstrate the importance of replicating landscape genetic analyses across populations and considering how landscape genetic effects may vary with spatial scale and local landscape structure.

KEYWORDS

bobcat, connectivity, fragmentation, gene flow, landscape genomics, urbanization

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1 | INTRODUCTION

Urban development causes habitat degradation and fragmentation (LaPoint, Balkenhol, Hale, Sadler, & van der Ree, 2015; Ramalho & Hobbs, 2012). Habitat fragmentation exposes organisms to edge effects such as increased anthropogenic disturbance or changes in interspecific interactions such as predator-prey relationships and competition (Fahrig, 2003; Murcia, 1995). Habitat fragmentation also isolates populations and reduces functional connectivity, defined as the degree to which the landscape facilitates or impedes movement among patches (Taylor, Fahrig, Henein, & Merriam, 1993). Isolated populations are susceptible to inbreeding depression and genetic drift that reduce overall fitness and adaptive potential in the face of current and future threats, such as climate change and novel pathogens (Hoffmann, Sgrò, & Kristensen, 2017; Keyghobadi, 2007), and suffer reduced potential for demographic rescue (Brown & Kodric-Brown, 1977). However, the consequences of fragmentation may vary among populations and species due to variation in factors such as patch size, the distribution and intensity of urban development, landscape characteristics such as topography and vegetation, as well as intrinsic factors such as species vagility or generality of habitat requirements (Johnson & Munshi-South, 2017; Rivkin et al., 2019). In addition, the impacts of habitat fragmentation and landscape drivers of connectivity may vary across spatial scales (Cushman & Landguth, 2010a; Vandergast, Bohonak, Hathaway, Boys, & Fisher, 2008; Vandergast, Bohonak, Weissman, & Fisher, 2007), particularly in urban environments (Miles, Dyer, & Verrelli, 2018). Connectivity studies of single landscapes have limited ability to account for this variation and are susceptible to over-generalization of findings that may only be locally relevant. Thus, replication across populations or landscapes is necessary to assess the generality of as well as the scale- and context-dependency of landscape influences on connectivity. However, except for some recent examples (Balbi et al., 2018; Miles et al., 2018; Robertson et al., 2018; Row et al., 2018; Short Bull et al., 2011), few connectivity studies incorporating such replication exist.

The impacts of urban development can be particularly acute for mammalian carnivores (Fuller, DeStefano, & Warren, 2010; Ordeñana et al., 2010; Randa & Yunger, 2006; Tracey et al., 2013). Many carnivore species are territorial, exist at low population densities, and require large, connected areas of habitat to support viable populations (Noss, Quigley, Hornocker, Merrill, & Paquet, 1996). Consequently, habitat fragmentation and its genetic and demographic effects are frequently implicated in carnivore declines (e.g., Beier, 1993; Dixon et al., 2007; Ernest, Vickers, Morrison, Buchalski, & Boyce, 2014; Lu et al., 2001; Roelke, Martenson, & O'Brien, 1993). Given their sensitivity to fragmentation, carnivores are excellent indicator species of functional landscape connectivity (Hunter, Fisher, & Crooks, 2003; Noss et al., 1996). Moreover, their large home ranges frequently make them useful umbrellas for conserving broader ecological communities (Noss et al., 1996). These factors, in addition to the potentially important role that carnivores can play in ecosystems (Estes et al., 2011), particularly in constrained urban landscapes (Crooks, Riley, Gehrt, Gosselink, & van Deelen, 2010; Crooks & Soulé, 1999), emphasize the value in understanding how landscape influences carnivore connectivity.

Coastal southern California is one of the most urbanized landscapes in North America, having experienced rapid human population growth and expansion of developed areas over the past several decades (U.S. Census Bureau, 2010), with a human population of over 13.3 million in the Los Angeles metropolitan area alone (U.S. Census Bureau, 2016). This region is also renowned as a hotspot for biodiversity and endemism, with habitat fragmentation and loss leading to high concentrations of threatened species in remaining natural areas (Dobson, Rodriguez, Roberts, & Wilcove, 1997; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). Despite extensive urban development, coastal southern California retains relatively intact communities of carnivore species that vary in their requirements for habitat patch size and quality, and in their overall sensitivity to urbanization (Crooks, 2002; Ordeñana et al., 2010). In particular, bobcats (*Lynx rufus*) are the third largest carnivore in coastal southern California, having intermediate sensitivity to urbanization and a requirement for adequate linkages among habitat patches (Crooks, 2002; Riley et al., 2003). Consequently, the status of bobcat populations is regarded as an important indicator of functional landscape connectivity in this region (Crooks, 2002; Hunter et al., 2003; Tracey et al., 2013).

Telemetry studies show that bobcats in coastal southern California rely on natural areas that consist predominantly of coastal sage scrub and chaparral vegetation (Riley et al., 2003; Tracey et al., 2013). However, bobcats are also habitat generalists and may persist near to and even within anthropogenically altered and populated areas (Hunter et al., 2003; Lyren, Alonso, Crooks, & Boydston, 2008b; Riley et al., 2003). Although major roads and dense urban development are barriers to functional connectivity in this region, telemetry and genetic studies (including pathogen genetics) have indicated occasional crossing of major roads by bobcats, mostly facilitated by culverts or underpasses (Fountain-Jones et al., 2017; Lee et al., 2012; Lyren et al., 2008b; Poessel et al., 2014; Riley et al., 2006; Serieys, Lea, Pollinger, Riley, & Wayne, 2015). Nonetheless, several independent microsatellite studies have broadly characterized a collection of genetically distinct bobcat populations, which

are confined to discrete habitat patches of varying size separated by major roads and areas of concentrated urban development (Lee et al., 2012; Riley et al., 2006; Ruell et al., 2012; Serieys et al., 2015; Thomassen et al., 2018). These studies have focused on hard anthropogenic barriers influencing connectivity among these populations, and thus the factors driving connectivity within populations are generally unknown. However, we might expect these drivers to vary from one population to another due to variation in patch size and degrees of urban association in this region.

Landscape factors influencing connectivity probably differ within versus between populations because these factors have often contributed to the formation of population boundaries themselves. Therefore, the study of how landscape factors within populations influence connectivity can provide different insights compared to the study of how landscape influences connectivity among populations. Furthermore, by narrowing our focus to populations and patches contained within the bounds determined by hard anthropogenic barriers such as highways (thereby excluding the strong genetic signals of these barriers from analysis), we can assess finer scales of both spatial genetic variation and landscape heterogeneity. Investigating this finer-scale variation can enable us to better detect which factors might be important for maintaining the degree of connectivity that necessarily must exist within a population, and to assess the potential impacts of landscape change on that connectivity (Cushman & Landguth, 2010a). Thus far, it remains unclear which specific natural features, if any, are important in maintaining bobcat genetic connectivity.

Next-generation sequencing technologies have greatly enhanced our ability to accurately estimate neutral genomic variation compared to microsatellites (Fischer et al., 2017; Helyar et al., 2011; Santure et al., 2010). Coupling genomic data with rigorous landscape genetic approaches that incorporate the individual (as opposed to the population) as the statistical unit provides considerable power for identifying genetic variation at fine spatial scales to quantify functional connectivity (Cushman & Landguth, 2010b; Holderegger & Wagner, 2008; Manel, Schwartz, Luikart, & Taberlet, 2003). However, individual-based connectivity studies and those using high-resolution estimates of gene flow have been relatively rare in urban landscapes (LaPoint et al., 2015). These tools are particularly valuable for studying connectivity in wide-ranging organisms such as bobcats, for which the influence of landscape on connectivity can be relatively subtle and difficult to detect. In addition, the spatial structure of bobcat populations in coastal southern California is well suited to a replicated landscape genetic design that tests factors affecting functional connectivity at multiple spatial scales across several comparable but distinct landscapes, with varying habitat structure and degrees of urbanization (Ruell et al., 2012).

We first aimed to identify landscape factors either promoting or constraining bobcat dispersal in coastal southern California, and secondly, to understand how these factors vary within and among populations from habitat patches with different landscape characteristics, including varying degrees of urbanization. We used next-generation sequencing to genotype bobcats at 13,520 SNP loci to make precise measurements of genetic relatedness among individuals. We then implemented an individual-based landscape genomic approach, testing support for landscape resistance variables representing possible effects of different landscape factors on bobcat connectivity, which we replicated among populations. Using this replicated landscape genomic framework, we test our first hypothesis: that factors influencing connectivity will vary among populations depending on spatial scale and patch-specific landscape context such as the extent of urbanization. Then, by analyzing individuals from all populations together, we test our second hypothesis: that factors affecting connectivity differ among versus within populations.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We utilized 286 bobcat blood and tissue samples derived from three previous studies conducted in different areas in southern California (Figure 1). San Diego samples (n = 43) were collected between 2007 and 2012 according to Jennings and Lewison (2013). Northwest Los Angeles (LA) samples (n = 133) were collected between 1997 and 2011 according to Riley et al. (2006) and Serieys et al. (2015). Southeast LA samples (n = 110) were collected between 2002 and 2010 according to Lyren et al. (2006), Lyren, Alonso, Crooks, and Boydston (2008a), Lyren et al. (2008b). All animals were sampled from a combination of live trapping (n = 258) and opportunistically collected carcasses (n = 28) of predominantly roadkill. Live animals were captured, handled, and released using protocols approved by cooperating agencies and relevant animal ethics committees (see original publications, cited above, for detailed information).

2.2 | Laboratory procedure

We extracted genomic DNA using the Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc.), eluting DNA in buffer EB. Agencourt Ampure XP SPRI beads (Beckman Coulter Inc.) were used to concentrate some low-yield DNA extractions. We prepared double-digest restriction-site-associated DNA (ddRAD) libraries according to Peterson, Weber, Kay, Fisher, and Hoekstra (2012), using *Nla*III and *Eco*RI-HF restriction enzymes, on individual samples normalized on a within-library basis to at least 200 ng DNA in 25 µl. A fragment size of 300–380 bp (excluding 75 bp adapters) was selected using a Blue Pippin size selection system (Sage Science Inc.) with a 100–600 bp 2% agarose gel cartridge containing internal standards, with fragment size verified using an Agilent Tapestation 2,200 (Agilent). A total of 48 uniquely barcoded P1 adapters enabled subsequent identification of pooled individuals, with biotinylated P2 adapters enabling streptavidin Dynabead (Invitrogen) purification to maximize efficiency of the final PCR amplification step. PCR was conducted over 12 cycles across five reactions per pool of individuals, using uniquely indexed primers to enable identification of each individual according to the pool of which it was a part, creating a two-tiered barcode or index system. This per-pool indexing allowed individual

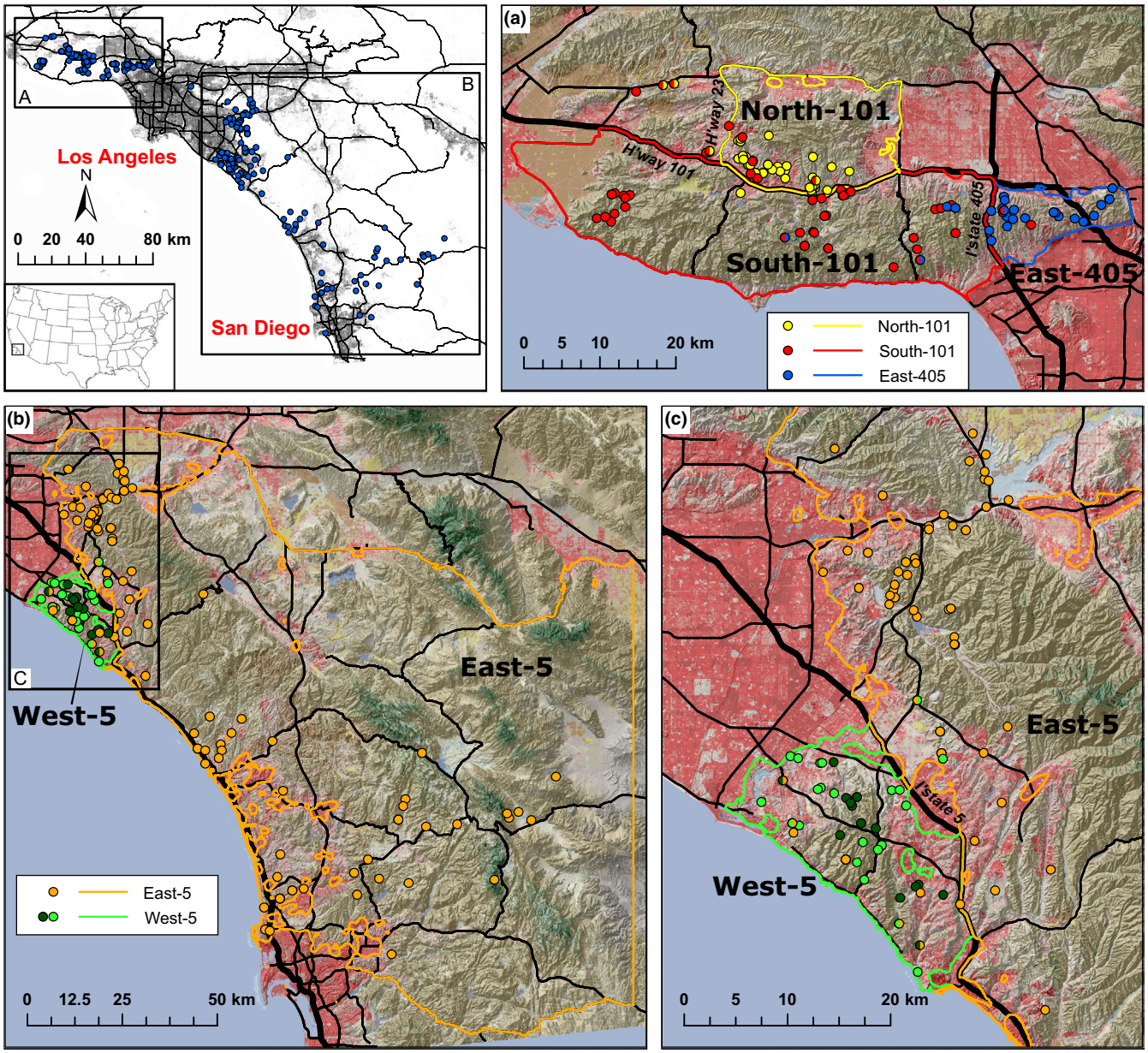

FIGURE 1 FASTSTRUCTURE analysis indicates five bobcat populations genetically isolated by geographic barriers in coastal Southern California, USA. All sampled individuals are shown as blue circles in the region-wide black-and-white map, with shading indicating urban development. Insets A–C indicate populations defined for landscape genomic analyses. Population spatial boundaries are indicated as solid coloured lines, and sample locations are coloured according to individual population assignment based on FASTSTRUCTURE analysis at $K = 3$ for both northwest and southeast of Los Angeles (Figure 2). Inset A shows populations northwest of Los Angeles, with East-405 ($n = 26$) indicated in blue, South-101 ($n = 43$) indicated in red, and North-101 ($n = 61$) indicated in yellow. Insets B and C show populations southeast of Los Angeles, with East-5 ($n = 97$) indicated in orange and West-5 ($n = 44$) indicated in light green and dark green. Individuals with greater than 25% admixture are shown with multiple colours. Highways are shown in all maps as black lines, with primary barriers to host gene flow (highway 101, Interstate 405, and Interstate 5) indicated by thick black lines. Topography and land cover are also shown in colour maps, indicating urbanization in pink/red, forests in green, grasslands and scrub in tan, and agricultural areas in brown [Colour figure can be viewed at wileyonlinelibrary.com]

P1 barcodes to be used more than once among multiple pools and combined within the same library.

An initial trial library of 16 individuals was prepared, followed by three libraries of 80 individuals, and one library of 82 individuals, multiplexed to obtain a minimum average sequencing coverage of 12× per locus, per individual. To enable estimation of sequencing error rates for optimization of locus assembly parameters (Mastretta-Yanes et al., 2015), detailed below, each of the prepared libraries contained five within-library replicates and five replicates shared with other libraries, except for the 16 sample library, which contained two individuals shared with other libraries/lanes. Collectively, a total of 12 individuals were replicated between libraries and 20 were replicated within libraries, among 306 unique individuals (including re-runs of 20 individuals due to initially low

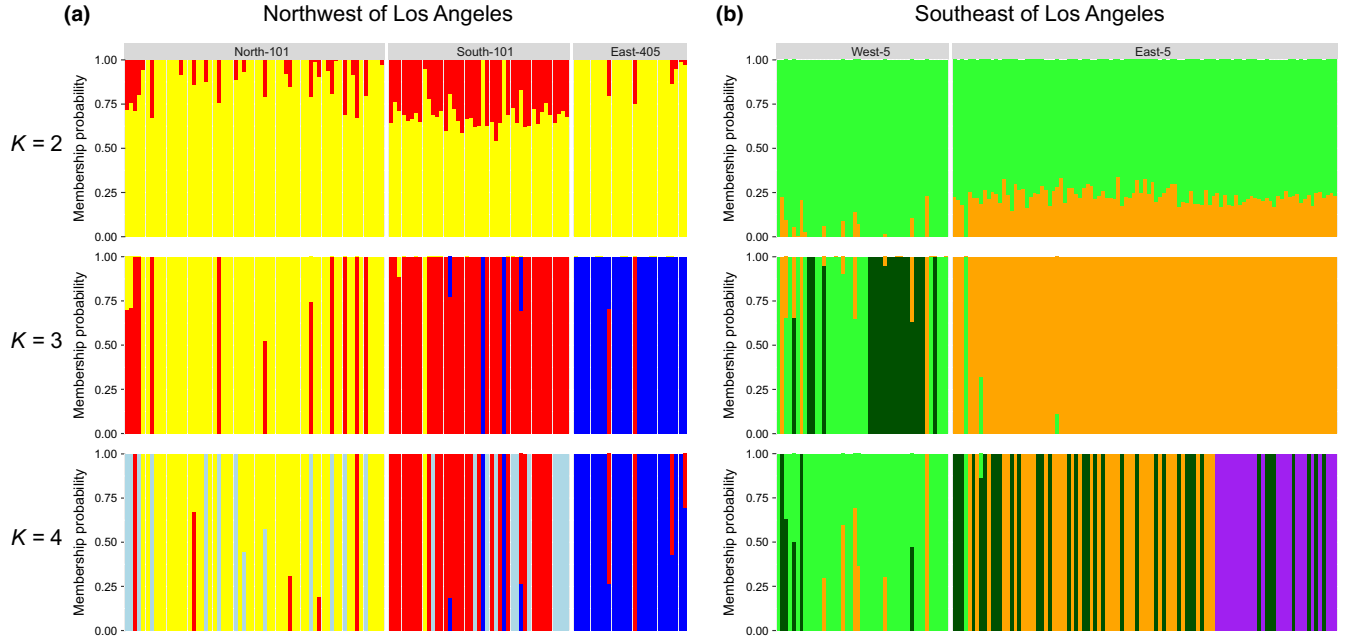

FIGURE 2 FASTSTRUCTURE analysis indicates population genetic structure among bobcats sampled from (a) northwest of Los Angeles and (b) southeast of Los Angeles, for K = 2, 3, and 4. These findings suggest three spatially and genetically distinct populations northwest of Los Angeles, and two spatially and genetically distinct populations southeast of Los Angeles. Individuals are organized along the x-axis according to distance from the boundary of the nearest neighbouring population, with individuals from North-101 in order of decreasing distance from South-101, individuals from South-101 in order of increasing distance from North-101, and individuals from East-405 in order of increasing distance from South-101. Individuals from West-5 are organized in order of decreasing distance from East-5, and individuals from East-5 are organized in order of increasing distance from West-5 [Colour figure can be viewed at wileyonlinelibrary.com]

sequencing coverage). Sequencing was conducted at the University of Oregon Genomics & Cell Characterization Core Facility for 100 bp, single-end reads, firstly on an Illumina Hi-Seq 2500 (Illumina) for the 16 individual and one 80 individual library, and on an Illumina Hi-Seq 4000 for the subsequent 80 and 82 individual libraries. Each library was sequenced on a separate lane.

2.3 | Bioinformatics and data filtering

Raw sequence files were initially checked for quality using FastQC (Andrews, 2010). Stacks version 1.42 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013) was then used to assemble reads into loci and identify single-nucleotide polymorphisms (SNPs). Per-individual demultiplexing of sequencing reads, Phred score quality filtering, and trimming of adapters was performed using the Stacks program process_radtags (http://catchenlab.life.illinois.edu/stacks/). The Stacks pipeline for nonreference-aligned data, DENOVO _ MAP.PL, was used to build loci and identify SNPs from stacks of sequence reads, populate a catalogue containing sets of consensus loci, and match individuals against the catalogue to call alleles at each locus for each sample.

Four user-specified parameters have been shown to influence rates of error introduced during DENOVO _ MAP.PL, with optimal settings being specific to each data set (Mastretta-Yanes et al., 2015; Paris, Stevens, & Catchen, 2017): the minimum number of identical, raw reads required to create a stack (*-m*); the maximum number of mismatches allowed between loci when processing a single individual (*-M*); the maximum number of mismatches allowed between loci when building the catalogue (*-n*); and the maximum number of stacks at a single de novo locus (*-max_locus_stacks*). Using between- and within-library replicates, we conducted 11 trials of DENOVO _ MAP.PL, varying a single parameter at a time, and calculated locus error (proportion of loci genotyped in only one of a pair of replicate individuals), allele error (proportion of allele mismatches among replicate pairs per locus), and SNP error (proportion of SNP mismatches among replicate pairs) for each trial according to Mastretta-Yanes et al. (2015).

DENOVO _ MAP.PL was run using the full data set with parameter settings chosen to minimize error and maximize the number of SNP loci (*-m* = 3, *-M* = 2, *-n* = 4, *-max_locus_stacks* = 3). We generated a SNP matrix containing allele calls for each individual using the Stacks program populations with minimal filtering, except to retain only loci that were present in >20% of individuals in each population (defined in this step as northwest LA, southeast LA, and San Diego populations, as above), and only a single, randomly chosen SNP per locus. We used PLINK version 1.07 (Purcell et al., 2007) for further filtering of the SNP matrix. Loci missing from >35% of individuals were removed, followed by individuals missing >50% of loci, and loci with a minor allele frequency <0.01. A disproportionate number of SNPs were located at read positions 94 and 95, indicating increased sequencing error at these positions; these SNPs were also removed.

TABLE 1 Landscape resistance variables tested

Landscape variable	Description	Data source and original raster resolution	Landscape resistance hypothesis	Ecological justification
Isolation by distance (IBD)	Null model representing an isolation by distance effect	N/A	Homogeneous resistance surface; every cell has a resistance of 1	Genetic differentiation increases with geographical distance (Wright, 1943)
Impervious surface (IMPERV)	Measure of urban density, taken as the percentage landcover of impervious surfaces (e.g. buildings, concrete) per cell	2011 National Land Cover Database (mrlc.gov/nlcd2011.php; Homer et al., 2015) 30 m	Resistance increases with increasing % impervious surface. Linear relationship 1–100	Bobcats have been shown to avoid urban areas (Lyren et al., 2008b; Ordeñana et al., 2010; Riley et al., 2003)
Roads (ROAD)	All major and minor roads	OpenStreetMap (http://download.geofabrik.de/north-america/us/california.html) 30 m	All cells within 30 m of a road have high resistance (100), all other cells have low resistance (10)	Bobcats prefer habitat containing fewer roads (Poessel et al., 2014)
Streams (STREAM)	All linear surface water features, including perennial, intermittent, and ephemeral streams, canals, and artificial channels	National Hydrography Data set (nhd.usgs.gov) 30 m	All cells with 50 m of a stream have low resistance (10), cells within 75 m of a stream have moderate resistance (50), cells within 100 m have moderate-high resistance (75), and all other cells have high resistance (100)	Bobcats favour riparian corridors for dispersal (Hilty & Merenlender, 2004; Jennings & Zeller, 2017)
Topographic roughness (ROUGH)	Unitless index representing the amount of elevation variation with a 3-by−3 cell moving window, calculated using Geomorphometry & Gradient Metrics Toolbox (Evans, Oakleaf, Cushman, & Theobald, 2014)	National Elevation Data set (lta.cr.usgs.gov/ned) 10 m	Cells with higher topographic roughness have lower resistance; transformed from raw values to resistances from 1–100 using the 'MSSmall' function[a]	Carnivores are known to use topographic features as movement corridors (Lee & Vaughan, 2003; Dickson & Beier, 2007)
Vegetation density (VEG)	Enhanced Vegetation Index calculated from chlorophyll reflectance satellite imagery, measured in 2016	Moderate Resolution Index Imaging Spectroradiometer (modis.gsfc.nasa.gov) 250 m	Cells with higher vegetation density have lower resistance; transformed from raw values to resistances from 1–100 using the "Small" function[a]	Bobcats more often occur in natural, vegetated habitats (Ordeñana et al., 2010)

[a]Transformation functions implemented using the "Rescale by Function" tool in ARCGIS (ESRI).

TABLE 2 Models for comparing individual pairwise genetic distances (D_{ps}) to landscape resistance distances in each population

Model name	Model parameters
FULL	D_{ps} ~ ROUGH + VEG + IMPERV + ROAD + STREAM
HABITAT	D_{ps} ~ VEG + IMPERV + STREAM
DEVELOP	D_{ps} ~ IMPERV + ROAD + ROUGH
LINEAR	D_{ps} ~ ROAD + STREAM
UNIV.IMP	D_{ps} ~ IMPERV
UNIV.VEG	D_{ps} ~ VEG
UNIV.RD	D_{ps} ~ ROAD
UNIV.STRM	D_{ps} ~ STREAM
UNIV.TR	D_{ps} ~ ROUGH
IBD	D_{ps} ~ IBD

Principal components analysis revealed a batch effect pertaining to our 80-sample library sequenced on the Illumina Hi-Seq 2500, which produced lower average coverage than our other libraries. This was corrected using an R (R Development Core Team, 2013) script employing functions from the ADEGENET (Jombart, 2008) and POPPR (Kamvar, Brooks, & Grünwald, 2015) packages to compare rates of missing data for each locus per library, and remove loci for which the missing data rate in any library was above or below 1.5 times the interquartile range (as calculated from the missing data rates for a given locus across all libraries). We used PCADAPT (Luu, Bazin, & Blum, 2017) to identify outlier loci potentially under selection, with *Q*-value false discovery rate correction (α_Q = 0.10). Following filtering, populations in Stacks was re-run using SNP-specific whitelists to produce final matrices containing putatively neutral SNPs that passed filtering for use in subsequent population and landscape genetic analyses.

2.4 | Population genomic structure

We employed the SNP data set to define the populations on which subsequent landscape genetics would be based. Although five genetically-distinct populations have been previously defined in our study region based on microsatellite variation, we refined these boundaries using our SNP data set given expectations of slight differences owing to the markers and individuals analyzed between studies. Patterns of neutral genomic variation were initially visualized using principal components analysis in R. We used FASTSTRUCTURE (Raj, Stephens, & Pritchard, 2014) with no a priori population assignments to simultaneously estimate the number of genetic populations (*K*) and probabilistically assign individuals to populations based on their multilocus genotypes. The FASTSTRUCTURE script CHOOSEK.PY was run using simple priors and five-fold cross validation to select a range of plausible *K* values based on maximizing marginal likelihood (tends to underestimate *K*; Raj et al., 2014) and choosing the minimum number of populations that have a cumulative ancestry contribution of at least 99.99% (tends to overestimate *K*; Raj et al., 2014). These *K* estimates were subsequently verified by examining cross validation errors and comparing spatial patterns of individual population assignments with geographical features that may act as barriers to gene flow (e.g., major roads). To improve accuracy of individual population assignment probabilities, FASTSTRUCTURE was re-run for the identified plausible *K*-values using more computationally intensive logistic priors. Plots of FASTSTRUCTURE results showing individual assignment probabilities were constructed using ggplot2 (Wickham & Chang, 2008). Migrants were defined as having >50% FASTSTRUCTURE assignment probability to a population other than that in which the individual was sampled.

Population genetic statistics were estimated for each of the genetically defined populations. Observed and expected heterozygosity and *F*-statistics were calculated using DIVERSITY (Keenan, Mcginnity, Cross, Crozier, & Prodöhl, 2013). Allelic richness (A_r) was calculated using HP-RARE (Kalinowski, 2005) using the rarefaction procedure to account for differences in sample size among populations. The rarefaction sample size was set according to the population with the smallest sample size and was calculated as twice the number of individuals in this population genotyped for a given locus, averaged across all loci (we doubled the number of individuals as there were two alleles per locus). Effective population size (N_e) was calculated using the linkage disequilibrium method in NEESTIMATOR (Do et al., 2014) while controlling for the number of chromosomes to correct for downward bias in N_e estimates resulting from linkage within chromosomes (Waples, Larson, & Waples., 2016). We also excluded migrants (defined as above) from N_e estimates as these can introduce linkage disequilibrium bias (Waples & England, 2011). Pairwise genetic distances among populations (F_{ST}) were calculated using DIVERSITY with 95% confidence intervals calculated using 1,000 bootstraps carried out over individuals within populations.

We interpret relative values of genetic relatedness among individuals as being a product of gene flow and thus functional connectivity. Therefore, to quantify variation in connectivity within populations as well as across the entire study region, we measured genetic distances between pairs of individuals. Pairwise matrices of individual genetic distance were calculated as the inverse of the proportion of shared alleles (D_{ps}) using adegenet, both for each population individually as well as the entire data set.

2.5 | Landscape genomics

We used a landscape resistance framework to test hypotheses regarding the influence of landscape features within and among genetically-defined populations (see above) on gene flow (Table 1). This isolation-by-resistance analysis framework was chosen because we believe that in this highly urbanized environment, loss of connectivity among individuals and populations is the most likely mechanism by which landscape heterogeneity is affecting variation in gene flow. Alternatively, evolutionary or behavioural adaptation to local conditions may produce an isolation-by-environment effect whereby gene flow is reduced among populations adapted to different conditions (Wang & Bradburd, 2014). However, we show in a supplementary

analysis that variation in local conditions is unlikely to be affecting gene flow in these populations (Appendix S5).

We used ARCGIS 10.3 (ESRI) for all manipulation and analysis of spatial data except where specified otherwise. First, we demarcated the landscape area associated with each genetically-defined population according to major landscape features surrounding individuals assigned to the same population. Where a freeway or major highway (i.e., listed on the California State Highway network; Caltrans, 2017) passed near sampled individuals, and there were no individuals from the same genetic population sampled on the opposite side of the highway (with the exception of emigrants), this was used to define the spatial edge for a given population. However, where the edge of functionally impervious (defined below) urban development formed a continuous barrier between sampled individuals and the nearest major highway, this was instead used as the population edge. Functionally impervious urban development was defined using a method adapted from Ruell et al. (2012), by calculating for each 30 m by 30 m raster cell whether a majority of surrounding cells within a 1 km radius contained medium or high density urban landcover according to the National Landcover Database (Homer et al., 2015). This threshold was chosen based on previous work indicating that bobcats avoid areas of high urban intensity (Ordeñana et al., 2010; Riley et al., 2003; Tigas, Van Vuren, & Sauvajot, 2002). In one population (East-5) where there was no clear geographic barrier with which to define one edge, we defined the edge using a minimum bounding rectangle around all sample locations with a 20 km buffer. We believe this 20 km buffer is sufficient to capture any landscape that might be utilized for dispersal among our sample locations, but we acknowledge that quantification of landscape characteristics within areas defined in this way is imprecise. Previous research indicates that despite occasional short incursions, bobcats rarely make substantial movements through heavily urbanized areas (Lyren et al., 2008b; Riley et al., 2003). However, to account for the occasional use of these areas, and to eliminate any artificial edge effects in the landscape resistance analyses, we expanded each population area by one kilometer outside of the limits defined above, except where coastline formed the edge. We quantified the landscape characteristics of each genetic population within the areas defined above using Geospatial Modelling Environment (www.spatialecology.com).

For each of the defined genetic populations, we constructed landscape resistance surfaces representing hypothesized effects of landscape variables on bobcat gene flow for fine-scale landscape genetic analyses. Landscape variables hypothesized to be positively related to gene flow were topographic roughness, vegetation density, and streams, while urbanization and roads were hypothesized to be negatively related to gene flow. We rescaled raw landscape layers to a common scale of landscape resistance up to a maximum resistance of 100 as follows. Each categorical resistance surface (roads, rivers) was rescaled so that the minimum resistance = 10, and the maximum resistance = 100. Resistance surfaces representing continuous variables (topographic roughness, vegetation, urbanization) were rescaled to values between 1 and 100. In addition, we conducted transformations of the resistance surfaces using ARCGIS where we believed there was a clear ecological justification. For example, the topographic roughness and vegetation density resistance surfaces were transformed following our observations that the linearly transformed raw data did not adequately reflect the degree of heterogeneity that would be experienced by bobcats on the landscape (due to large skews in the raw data such as where a single cell containing a tall cliff would have a topographic roughness of orders of magnitude higher than the median, compressing most of the variation in the landscape into a very small range of resistance values). Although we did not exhaustively test all possible transformations, we chose transformations that best reflected our a priori biological hypotheses about how a given variable might affect bobcat movement (Table 1). We also generated an undifferentiated resistance surface for each population, representing isolation by distance (IBD; null model). We resampled all population-specific resistance surfaces to ensure a consistent resolution of 30 m by 30 m. For the study-wide landscape genetic analysis, we constructed resistance surfaces encompassing the entire study area, which we resampled to a resolution of 60 m by 60 m due to computational constraints. Preliminary tests across a smaller area produced highly similar results among 30 m and 60 m resolutions. For each resistance surface, spatial data sources, resistance surface parameterization (including transformations where applicable), and ecological justifications are presented in Table 1. CIRCUITSCAPE 4.0.5 (McRae, Dickson, Keitt, & Shah, 2008) was used to model connectivity between individuals across each resistance surface to produce pairwise matrices of hypothesized landscape resistance to gene flow among individuals. CIRCUITSCAPE was run for each population individually, as well as across the entire region at once. Migrants were removed from single-population landscape genetic analyses because genetic differentiation due to among-population barriers could not be adequately accounted for in our single-population resistance surfaces (see Appendix S4 demonstrating that model performance was substantially lower with migrants included). However, migrants were retained for the whole region ("study wide") analysis.

Associations between landscape resistance matrices and pairwise genetic distances were tested using linear mixed effect models incorporating a maximum likelihood population effects (MLPE) approach (Clarke, Rothery, & Raybould, 2002; Van Strien, Keller, & Holderegger, 2012) using the LME4 package in R. This method incorporates a random effect structure that accounts for the nonindependence among pairwise data, and has been shown recently to outperform other model selection methods for landscape genetics (Shirk, Landguth, & Cushman, 2018). Prior to fitting models, matrices of D_{ps} were log-transformed to satisfy normality assumptions and all dependent and independent variables were rescaled to units of standard deviation and a mean of zero. Ten models were fitted per population, including four multivariable models and all six possible single-variable models (Table 2). Multivariable models were built according to general hypotheses about how gene flow might be influenced by landscape, an approach which is favoured over testing all possible multivariable models (Burnham & Anderson, 2002). For example, the "HABITAT" model explored the hypothesis that the

distribution of habitat in the form of vegetation is driving gene flow patterns and included as fixed effects vegetation density, streams (accounting for increased riparian vegetation density), and impervious surfaces (urban areas contain less habitat). Other multivariable models were FULL (all fixed effects included), DEVELOP (the distribution of anthropogenic development is driving gene flow; includes topographic roughness, which has influenced where development has occurred), and LINEAR (linear features, i.e., roads and streams, act as barriers to or corridors for gene flow). Multicollinearity among fixed effects were assessed for each multivariable model by calculating the variance inflation factor (VIF). Variables with VIF > 10 were considered highly correlated and were excluded from final models.

MLPE models were initially fitted and evaluated for each population using the Bayesian Information Criterion (BIC), which Row, Knick, Oyler-McCance, Lougheed, and Fedy (2017) found to outperform R^2 for ranking models. All models with a ΔBIC < 5 were considered candidates for the best model. Marginal R^2 were calculated for descriptive purposes (calculated using the MUMIN package; Bartoń, 2014). Well-supported candidate models were then refitted using restricted maximum likelihood (REML) for unbiased estimation of beta coefficients (Clarke et al., 2002; Van Strien et al., 2012). Row et al. (2017) found that inclusion of an undifferentiated resistance variable representing IBD was effective in factoring out the effect of distance in MLPE models and reduced the likelihood of type I error in estimating landscape resistance variable significance. Therefore, we also included an IBD fixed effect to all candidate models to improve accuracy of beta coefficient estimates. We calculated upper and lower 95% confidence intervals (CIs) of beta coefficients for all variables in REML-refitted candidate models. Model averaging of variable beta coefficients was conducted for each population using BIC evidence weights. Variables that had positive beta coefficients with CIs that did not overlap zero were considered to have a significant effect. Using this approach, landscape resistance variables with significant negative beta coefficients typically indicate a nontrue relationship (Row et al., 2017) and were thus interpreted here as nonsignificant.

3 | RESULTS

3.1 | Genotyping and data filtering

Minimal variation in SNP numbers and error rates (locus, allele, and SNP) was observed among replicate samples due to changes in the DENOVO _ MAP.PL parameter settings. Nonetheless, optimal parameter settings were chosen according to these measures, resulting in an average locus error rates of 0.289, an average allele error rate of 0.066, and an average SNP error rate of 0.027 prior to filtering of the SNP matrix. Initial processing of raw sequencing data in Stacks using optimal parameter settings resulted in a matrix of 141,705 SNPs among 286 individuals. Following filtering of individuals and loci for missing data, and of loci for low minor allele frequencies, outlier loci, and exclusion of SNPs at read positions 94 and 95, a final matrix of 13,520 SNPs among 271 individuals remained for analysis of population genomic structure (see Table S1.1 for detailed filtering results).

3.2 | Population genomics

Analyses of population genomic structure using FASTSTRUCTURE indicated distinct genetic clusters northwest and southeast of Los Angeles, which were further structured with respect to certain major roads. For the northwest of LA group (Figure 1a), K = 2–3 was identified as optimal, with cross-validation error being lowest at K = 3 (Figure 2a). We chose K = 3 as the most plausible number of genetic clusters based on these results and because the identified populations and subsequent individual assignment probabilities corresponded spatially with major geographical features (i.e., highways). These results indicated one genetic population located north of California State Highway 101 (this population subsequently referred to here as North-101), and two other populations that were both located south of Highway 101: one west of Interstate 405 (South-101) and one east of Interstate 405 (East-405).

For the southeast of LA group (Figure 1b,c), K = 1–3 was identified as optimal, with cross-validation error being lowest at K = 4 (Figure 2b). These analyses had difficulty consistently resolving spatial genetic groups in this region, with the only repeated delineation being among individuals sampled east and west of Interstate 5 (I-5). Within these two groups, some substructure was identified, but not consistently among runs at different K values. Thus, we determined that the southeast of LA group contained two genetic populations separated by I-5. These two clusters consisted of individuals sampled from the San Joaquin Hills area west of I-5 (this population and the area it occupies are subsequently referred to as West-5), and one population east of I-5 (East-5) consisting of individuals across a large area in the Santa Ana Mountains south of Los Angeles and north of San Diego (Figure 1b). For quantifying rates of migration and admixture among West-5 and East-5, assignment probabilities for K = 3 appeared to be most informative, so we identified among-population migrants using this model. However, close scrutiny of K = 2 and K = 4 revealed support for the same migrant individuals. Substructure within the West-5 population detected with K = 3 did not appear to correspond to any spatial or temporal pattern. Substructure within East-5 was detected only at K = 4, with clusters spatially organized in a roughly north-to-south pattern. However, these clusters overlapped spatially and did not appear to correspond to any known geographical barriers, potentially indicating a clinal pattern of genetic variation (Figures S1.1, and Figures S1.2) driven by isolation-by-resistance relationships (please see Appendix S2 for an additional analysis that corroborates our FASTSTRUCTURE findings).

We detected 21 migrant individuals across the study area using FASTSTRUCTURE. These included 12 individuals located north of Highway 101 that genetically assigned to the South-101 population. A high proportion of individuals north of Highway 101 and west of Highway 23 had mixed ancestry (North-101, South-101 populations) and therefore the population assignment of this area is uncertain and was excluded from further study. The South-101 population

TABLE 3 Pairwise F_{ST} among each population pair (95% confidence intervals shown in parentheses), indicating varying degrees of genetic differentiation among populations

	East–5	West–5	East–405	South–101
West-5	0.040 (0.031–0.049)	–	–	–
East-405	0.087 (0.076–0.106)	0.123 (0.103–0.145)	–	–
South-101	0.039 (0.034–0.044)	0.073 (0.064–0.083)	0.058 (0.046–0.074)	–
North-101	0.049 (0.043–0.056)	0.085 (0.076–0.095)	0.091 (0.082–0.122)	0.035 (0.028–0.044)

area contained one individual that assigned to the North-101 population, and two individuals that assigned to East-405. The East-405 population area contained two individuals that assigned to the South-101 population. We identified less migration among the two populations located southeast of Los Angeles, with one individual located east of Interstate 5 assigned to West-5, and three individuals located west of I-5 assigned to East-5. Pairwise F_{ST} values were statistically significant among all populations and ranged between 0.041 and 0.150 (Table 3), supporting the genetic clusters identified using FASTSTRUCTURE.

Population East-405 was the least genetically diverse based on allelic richness and nucleotide diversity, with these measures also relatively low in West-5 (Table 4). East-5 and South-101 had the highest measures of allelic richness and nucleotide diversity but were also the largest populations based on geographic area. Effective population sizes were generally congruent with the genetic diversity measures, with populations with higher genetic diversity having higher effective population sizes (Table 4; for a detailed discussion of our population genomics findings, please see Appendix S3).

3.3 | Landscape genomics

Geographic extent and landscape composition varied among populations (Table 5). The population with the largest area was East-5 (15,067 km^2), with all other populations below 1,000 km^2. East-5 was also the least urbanized and had the fewest roads, with the highest degrees of urbanization and road density being in populations with the smallest spatial size, East-405 and West-5. Stream density was relatively consistent among populations, except for East-405 which had comparatively few streams. Topographic roughness was highest in the South-101 population, which encompassed the Santa Monica Mountains, and was lowest in West-5.

No collinearity was detected among predictors, except among the resistance distances for impervious surfaces and roads in the East-405 population; we thus excluded impervious surfaces from all multivariable models for East-405 (although we retained it for the single-variable model). For the study-wide landscape resistance analysis, linear mixed effects models with MLPE returned only the full model as a candidate for the best model according to BIC (Table 6). Within this model, we found significant effects of topographic roughness and impervious surfaces with roughness positively associated and impervious surfaces negatively associated with gene flow, but no significant effect of IBD, vegetation, roads, or streams (Table 6, Figure 3). The population inhabiting the largest area, East-5, showed significant effects of vegetation, impervious surfaces, and streams, but no effect of IBD, indicating that vegetation and streams were positively associated with gene flow, and impervious surfaces were negatively associated with gene flow.

Among the spatially smaller populations, generally fewer significant landscape effects on genetic distances were identified. We found strong effects of impervious surfaces on gene flow in both South-101 and East-405, which had relatively low densities of streams (Figure 4). Conversely, populations exhibiting evidence for streams being positively associated with gene flow (East-5, South-101, and North-101) had generally lower urbanization and road density and had larger areas (Figure 4). However, two of these populations showed only near-significant support for streams (South-101 and North-101; Figures 3 and 4). There was near-significant support for a negative effect of roads on gene flow in North-101 and South-101 (Figure 3). IBD was the only supported predictor of pairwise genetic distances in West-5. Marginal R^2 values

TABLE 4 Genetic diversity statistics for each population with the number of genotyped individuals used for calculating these statistics (n), allelic richness (A_r), observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}), inbreeding coefficient (F_{is}), and effective population size (N_e; calculated with nonmigrants only). The greatest genetic diversity and effective population sizes were observed in East-5 and South-101, while East-405 contained the lowest diversity and smallest effective population size

Population	n	A_r	H_{obs}	H_{exp}	F_{is}	N_e
North-101	61	1.75	0.1533	0.1902	0.1693	22.9 (16.4–33.6)
South-101	43	1.79	0.1640	0.1940	0.1252	90.3 (46.3–504.2)
East-405	26	1.62	0.1408	0.1679	0.1300	12.8 (6–35.1)
West-5	44	1.71	0.1624	0.1854	0.1132	18.9 (14.4–25.5)
East-5	97	1.83	0.1513	0.1963	0.2069	150.3 (100.5–271.8)

TABLE 5 Landscape characteristics for each genetic population ± standard deviation, indicating substantial variation among populations. Linear features (roads and streams) are given as average length of features per square kilometer, with all other features given as the average raster cell value across the population area. Urbanization (impervious surface) are actual percentage values (i.e., 100% urbanization means that the impervious surfaces cover 100% of a raster cell), while vegetation density and topographic roughness are percentages relative to the highest and lowest values of each variable across the whole region

Population	Total area (km^2)	Vegetation density (%)	Urbanization (%)	Roads (km/km^2)	Streams (km/km^2)	Topographic roughness (%)	Elevation (m)
North-101	406	38.2 ± 7.27	16.9 ± 23.0	5.55	1.57	2.35 ± 2.49	348 ± 100
South-101	959	49.3 ± 12.2	9.45 ± 19.1	3.60	1.53	3.74 ± 4.01	280 ± 174
East-405	185	47.1 ± 11.5	28.8 ± 29.1	9.41	0.53	2.93 ± 3.37	225 ± 89.6
West-5	340	43.4 ± 10.2	27.7 ± 27.8	9.22	1.97	1.83 ± 2.11	98.1 ± 67.7
East-5[a]	15,067	38.4 ± 15.9	6.68 ± 17.5	2.43	1.52	2.62 ± 3.20	632 ± 445

[a]Landscape characteristics for this population are estimates only as it has a high degree of openness to external source populations without a clearly defined geographical boundary (e.g., major highways known to be acting as barriers).

were highest in the study-wide candidate model (mR^2 = 0.640; Table 6), East-5 (mR^2 = 0.318) and in East-405 (mR^2 = 0.269), and lowest in North-101 (mR^2 = 0.053–0.124), indicating substantial variation among populations in the ability of our models to explain individual genetic variation.

4 | DISCUSSION

4.1 | Effect of spatial scale on landscape genetic inference

Understanding how landscape features influence connectivity within and among populations, and hence at varying spatial scales, is important for identifying factors that maintain connectivity and to elucidate the impacts of habitat degradation on genetic variation (Johnson & Munshi-South, 2017; Miles et al., 2018; Rivkin et al., 2019). In most of our studied populations, a proportion of the variation in genetic distances among individuals was attributable to one or more of our landscape resistance hypotheses. However, the spatial scale over which these hypotheses were tested appeared to be an important factor, with greater proportions of genetic variation explained by our models at larger spatial scales (64% study-wide; 32% in the largest population; compared to generally <13% in smaller populations). The ratio of dispersal distance to study area has a substantial influence on the proportion of spatial genetic structure that is likely to be explained by landscape heterogeneity, with a greater proportion of variation explained when this ratio is lower. This is because species that disperse long distances exhibit spatial genetic variation over larger areas than those that disperse short distances. Thus, sampling over larger areas is required in order to detect patterns of variation in long-distance dispersers that reflect landscape effects. For example, in studies of species with very low dispersal distances compared to bobcats, landscape genetic models can explain upwards of 40% of genetic structure across study areas comparable in size to our smaller populations (Funk et al., 2005; Goldberg & Waits, 2010; Murphy, Evans, & Storfer, 2010; Vandergast et al., 2007; Wang, 2009).

We found strong support for models containing multiple significant effects of landscape variables in both the study-wide analysis and East-5. In contrast, we observed fewer effects of landscape resistance in the four populations with the smallest spatial size. Strong support for impervious surfaces restricting gene flow was evident in South-101 and East-405, but West-5 showed no support for any resistance hypothesis other than IBD, and there was only minimal statistical support for any resistance hypothesis in North-101. Our findings reflect those of other recent landscape genetic studies employing replicate populations or landscapes that demonstrate the scale dependency of many landscape genetic relationships (Balbi et al., 2018; Miles et al., 2018; Robertson et al., 2018; Row et al., 2018). Sample size did not appear to be a major factor affecting inferential power among our populations. Although we found higher support across larger spatial areas for landscape factors influencing gene flow, the smaller areas to which some populations were confined did not preclude us from detecting landscape genetic signals where connectivity was strongly impacted.

For the spatial scales at which habitat fragmentation is occurring in coastal southern California, bobcat populations are excellent indicators of functional connectivity (Crooks, 2002; Hunter et al., 2003). This is largely due to their intermediate sensitivity to anthropogenic disturbance and reliance on large, connected areas of natural habitat (Crooks, 2002; Ordeñana et al., 2010). Hard barriers such as highways or tracts of urban development can have a substantial and highly detectable effect on bobcat gene flow (Lee et al., 2012; Riley et al., 2006; Serieys et al., 2015; Thomassen et al., 2018). An effect of spatial scale on the detectability of environmentally-associated genetic variation has already been proposed in this region (Thomassen et al., 2018). However, by enhancing the detectability of this variation through high-resolution genomic data and landscape resistance analyses and by comparing across multiple spatial scales through multiple replicated analyses, our study provides further insights into the role of scale dependency in landscape genetic relationships. Within fully natural or altered natural areas, the high vagility and generalized habitat use of bobcats (Ordeñana et al., 2010; Riley, Boydston, Crooks, & Lyren, 2010) means that the effects of specific landscape factors on functional

TABLE 6 Linear mixed effect modelling with maximum-likelihood population effects indicates significant effects of landscape resistance variables on gene flow within genetic populations as well as across the entire region. Candidate models are listed according to ΔBIC (up to a maximum of five) calculated from initial fitting of models without restricted maximum likelihood (REML) and without the isolation by distance (IBD) fixed effect. Variance inflation factors (VIF) and marginal R^2 values reported are from these initial models. 95% confidence intervals for parameter beta coefficients (β) were calculated from refitting of candidate models with REML and IBD fixed effect included for enhanced accuracy and reduction of type I error. See Table 2 for model and parameter descriptions

						95% CI		
Population	Candidate models	ΔBIC	mR^2	Parameter	β	Lower	Upper	VIF
Region	FULL	0.00	0.64	Intercept	0.001	−0.385	0.387	
				IBD	−0.300	−0.557	−0.043	
				ROUGH[a]	**0.923**	**0.675**	**1.172**	6.07
				VEG	−0.219	−0.334	−0.103	2.88
				IMPERV[a]	**2.333**	**2.172**	**2.493**	2.90
				ROAD	−0.223	−0.404	−0.042	4.96
				STREAM	−0.435	−0.568	−0.302	3.48
North−101	UNIV.ROAD	0.00	0.058	Intercept	0.004	−0.236	0.244	
				IBD	0.021	−0.188	0.231	
				ROAD	0.201	−0.009	0.411	
	UNIV.TR	2.62	0.066	Intercept	0.005	−0.253	0.262	
				IBD	0.059	−0.290	0.407	
				ROUGH	0.177	−0.210	0.565	
	IBD	3.31	0.053	Intercept	0.005	−0.250	0.260	
				IBD[a]	**0.216**	**0.161**	**0.272**	
	UNIV.STRM	4.37	0.124	Intercept	0.005	−0.243	0.253	
				IBD	0.124	−0.012	0.260	
				STREAM	0.162	−0.057	0.380	
	LINEAR	4.72	0.068	Intercept	0.004	−0.225	0.233	
				IBD	−0.097	−0.335	0.141	
				ROAD[a]	**0.218**	**0.013**	**0.423**	1.25
				STREAM	0.177	−0.026	0.380	1.25
South−101	UNIV.IMP	0.00	0.075	Intercept	0.001	−0.371	0.374	
				IBD	−0.275	−0.666	0.117	
				IMPERV[a]	**0.514**	**0.116**	**0.913**	
	UNIV.RD	1.30	0.113	Intercept	0.001	−0.358	0.360	
				IBD	0.006	−0.245	0.256	
				ROAD	0.292	−0.033	0.616	
	UNIV.STRM	1.34	0.084	Intercept	0.000	−0.346	0.346	
				IBD	−0.140	−0.552	0.273	
				STREAM	0.396	−0.047	0.839	
	UNIV.TR	3.18	0.078	Intercept	0.001	−0.358	0.359	
				IBD	−0.228	−0.992	0.535	
				ROUGH	0.481	−0.325	1.286	
	IBD	3.96	0.071	Intercept	0.001	−0.336	0.337	
				IBD[a]	**0.226**	**0.177**	**0.275**	
East−405	UNIV.IMP	0.00	0.269	Intercept	0.006	−0.368	0.380	
				IBD	−0.635	−1.062	−0.208	
				IMPERV[a]	**1.090**	**0.664**	**1.515**	

(Continues)

TABLE 6 (Continued)

Population	Candidate models	ΔBIC	mR^2	Parameter	β	95% CI		VIF
						Lower	Upper	
West−5	IBD	0.00	0.125	Intercept	0.001	−0.258	0.260	
				IBD[a]	**0.345**	**0.265**	**0.425**	
East−5	HABITAT	0.00	0.318	Intercept	0.000	−0.249	0.248	
				IBD	−0.069	−0.219	0.082	
				VEG[a]	**0.348**	**0.199**	**0.498**	1.29
				IMPERV[a]	**0.209**	**0.038**	**0.379**	1.11
				STREAM[a]	**0.294**	**0.124**	**0.463**	1.35

*Indicates parameter significance, as determined by 95% confidence intervals greater than and not overlapping zero.

connectivity are often subtle. Thus, their detectability remains influenced by spatial scale, even with the high precision afforded by large genomic data sets.

4.2 | Implications for functional connectivity

Urban development containing impervious surfaces was the most frequently identified factor impacting connectivity, having a negative effect on gene flow largely irrespective of spatial scale or overall urban density. While bobcats generally occur more often in natural areas (Ordeñana et al., 2010), some telemetry studies show that bobcats will cross and sometimes utilize urban development separating habitat fragments, particularly at night (Riley et al., 2003; Tigas et al., 2002). Our results indicate that despite these movements, urban development does constrain gene flow even within areas that consist largely of natural habitat (e.g., East-5, South-101). The strongest effect of impervious surfaces appeared to be within our study-wide analysis, and due to urban development and major highways constraining gene flow among populations. At this broad scale, bobcat gene flow also favoured areas that were topographically rough, probably in part because such terrain precludes intensive urban development. This pattern is obvious in coastal southern California, where the largest tracts of urban development are situated in the flat regions of the Los Angeles Basin, coastal Orange County, and the coastal plain of San Diego County, and bobcat habitat generally is restricted to the surrounding Santa Monica Mountains, San Joaquin Hills, and eastern Peninsular Ranges. However, bobcats have been shown to also favour topographically rough terrain in less urbanized landscapes (Abouelezz et al., 2018). The ability to identify generalized effects such as that of urbanization here is a key advantage afforded by replication in landscape genetics generally (Balbi et al., 2018; Robertson et al., 2018; Row et al., 2018). However, where results differ among populations, a replicated design also provides an opportunity to explore what localized factors may have led to these differences.

Although we observed the effects of impervious surfaces in populations surrounded by relatively low urban development, we did not observe this pattern in some populations with moderate and high amounts of urban development (North-101 and West-5). As we outlined above, a lack of effect in these populations may be due to low detectability of landscape genetic signals at small spatial scales; however, variation among populations in dispersal behavior may be a contributing factor as well. Studies in other regions with different patterns of urban development have found that bobcats more strongly avoid urban areas in landscapes that are less fragmented by urbanization overall (Riley, 2006). In coastal southern California, bobcats in areas with relatively broad tracts of natural habitat may be better able to avoid urban areas through greater availability of alternative routes. Additionally, a home-range pileup effect has been observed previously in this region whereby territories adjacent to urban and highway barriers tend to be smaller and more densely distributed (Riley et al., 2006). As a result, juveniles from more urbanized areas may be required to disperse greater distances through potentially less suitable habitat (e.g. areas containing more impervious surface) to find territory. Nonetheless, a strong negative effect of impervious surfaces on gene flow was detected in our smallest and most heavily urbanized population (East-405). For such a small patch of habitat, it is concerning that the effect of impervious surfaces explained such a high proportion of the among-individual genetic variation within this population (mR^2 = 0.269). Further urban development in this area has a risk of producing smaller, more isolated habitat patches that may be insufficient to support viable bobcat populations, resulting in localized extinction. The area east of I-405 no longer supports a viable population of mountain lions due to urban habitat fragmentation (Riley et al., 2014), and our results suggest that bobcats are at risk of a similar fate.

In urban populations, the landscape factors that are responsible for maintaining gene flow are often poorly understood, despite their identification being critical for effective management of urban wildlife populations (Rivkin et al., 2019). Streams and vegetation had significant effects on gene flow only in East-5, our largest and least urbanized population. Vegetation is frequently found to favour bobcat movement and space use throughout the species' range (Abouelezz et al., 2018; Reding, Cushman, Gosselink, & Clark, 2013; Tucker, Clark, & Gosselink, 2008). In particular, the lack of an effect of vegetation in our more urbanized populations contrasts with a study of bobcats in Vermont, USA, which found that movement through vegetated areas is faster when in proximity to urban development compared to when the surrounding area was also vegetated (Abouelezz et al., 2018).

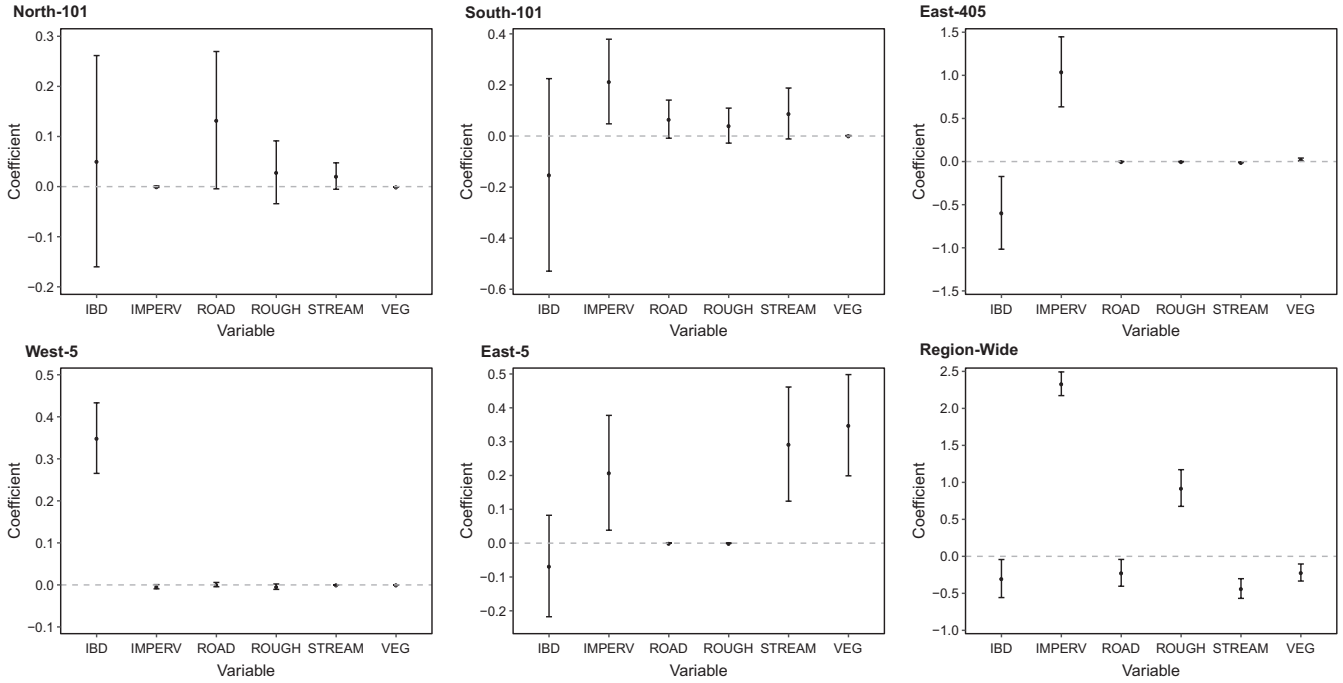

FIGURE 3 Average coefficients from MLPE candidate models indicate significant effects of landscape resistance variables on individual pairwise genetic distances among bobcats within five separate populations in coastal southern California, as well as among all individuals across the region. Mean coefficients are shown with upper and lower confidence intervals, weighted according to BIC model support. IBD, isolation by distance; IMPERV, impervious surfaces; ROAD, roads (major and minor) and highway links; ROUGH, topographic roughness; STREAM, all ephemeral, intermittent and perennial surface waterways; VEG, vegetation density

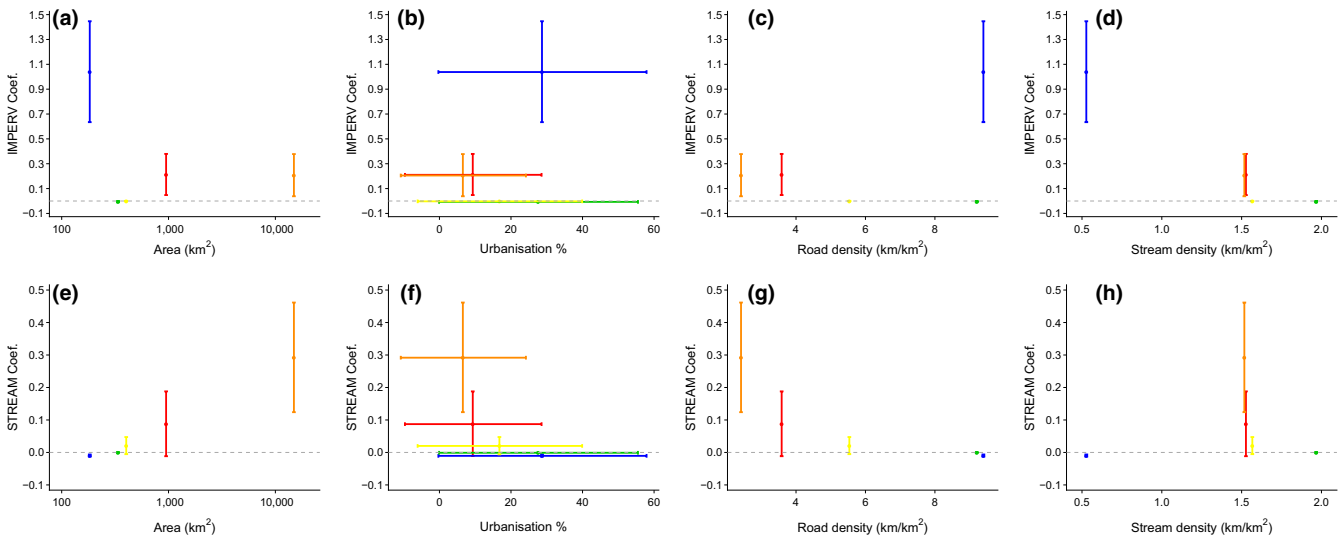

FIGURE 4 Trends in average beta coefficients for the effects of resistance distances representing impervious surfaces (a–d) and streams (e–h) on individual pairwise genetic distances in each population, relative to landscape characteristics of the area inhabited by each population. The effect of impervious surfaces on connectivity is generally greater in populations with fewer streams, while the effect of streams on connectivity is generally greater in populations encompassing a larger spatial area with lower urbanization and lower road density. Vertical error bars represent 95% confidence intervals; horizontal error bars represent ± 1 standard deviation. Colours indicate study population, with East-405 shown in blue, South-101 shown in red, North-101 shown in yellow, East-5 shown in orange, and West-5 shown in green. Comparisons between all combinations of resistance distance coefficients and population area characteristics are shown in Figure S1.3 [Colour figure can be viewed at wileyonlinelibrary.com]

In San Diego County, which encompasses a substantial part of our East-5 population, previous work has found associations between bobcat occurrence and water availability (Markovchick-Nicholls et al., 2008), as well as evidence for stream use as dispersal corridors (Jennings & Zeller, 2017). In addition, telemetry has indicated bobcats in the San Joaquin Hills utilize riparian corridors, particularly as

a means of traversing roads and urban areas (Lyren et al., 2008b), while camera surveys indicate bobcat use of riparian corridors in agricultural areas in northern California (Hilty & Merenlender, 2004). In contrast, our results suggest no overall effect of streams on gene flow in our San Joaquin Hills population (West-5), nor in East-405, our most heavily urbanized population. Again, such an effect may not be detectable at the relatively small spatial scales of these populations, and indeed, there was a weakly supported positive effect of streams in North-101 and South-101. Populations experiencing less urbanization and fewer roads appeared more likely to exhibit a positive effect of streams on gene flow (East-5 and South-101), while the strongest negative effect of urbanization on gene flow was in the population containing the fewest streams (East-405). One potential consequence of urbanization is the diversion of surface streams into channels and pipelines. This is particularly evident in the East-405 population, where the lower areas of many catchments contain suburban development with pipelines running underneath. The resulting loss of streams as usable habitat may explain why East-405 exhibited the highest effect of impervious surface in constraining gene flow (along with no positive effect of streams on gene flow).

In conclusion, our study demonstrates the value of multiple study areas and spatial scales when investigating the effects of landscape features on functional connectivity. Although our replicated design allowed us to draw generalized conclusions about the influence of specific landscape factors on gene flow (e.g., that of urbanization), no single factor had the same effect across all populations. This variability highlights the potential for single-population landscape genetic studies to miss important effects or overstate the generality of their findings. Further, variation among populations in landscape genetic relationships enables interpretation of these relationships with respect to variation in other underlying factors. Therefore, replication across populations and landscapes should be conducted wherever possible to properly assess the generality of landscape genetic effects and their dependence on spatial scale and landscape context.

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AUTHOR CONTRIBUTIONS

C.P.K. conducted the laboratory work, bioinformatics, statistical analysis, and wrote the manuscript with contributions from all authors. P.E.S., R.B.G., and D.R.T. provided input on the laboratory work, bioinformatics and statistical analyses. R.N.F., E.E.B., L.M.L., M.K.J., S.P.D.R., K.R.C., and L.E.K.S. collected samples. W.C.F., S.V., K.R.C., and S.C. conceived of the project. C.P.B., S.C., and W.C.F. oversaw the project and provided conceptual guidance.

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DATA AVAILABILITY STATEMENT

Demultiplexed sequencing reads and sample locations have been archived on Dryad, https://doi.org/10.5061/dryad.8pk0p2nhq. Kozakiewicz et al. 2019. All landscape data were obtained from publicly accessible sources, with links provided in Table 1.

REFERENCES

Abouelezz, H. G., Donovan, T. M., Mickey, R. M., Murdoch, J. D., Freeman, M., & Royar, K. (2018). Landscape composition mediates movement and habitat selection in bobcats (*Lynx rufus*): Implications for conservation planning. *Landscape Ecology*, *33*, 1301–1318. https://doi.org/10.1007/s10980-018-0654-8

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. Available from http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Balbi, M., Ernoult, A., Poli, P., Madec, L., Guiller, A., Martin, M. C., … Petit, E. J. (2018). Functional connectivity in replicated urban landscapes in the land snail (*Cornu aspersum*). *Molecular Ecology*, *27*, 1357–1370.

Bartoń, K. (2014). MUMIN: *Multi-model inference. R package version 1.10.0*. http://RForge.R-project.org/projects/mumin/

Beier, P. (1993). Determining minimum habitat areas and habitat corridors for cougars. *Conservation Biology*, *7*, 94–108. https://doi.org/10.1046/j.1523-1739.1993.07010094.x

Brown, J. H., & Kodric-Brown, A. (1977). Turnover rates in insular biogeography: Effect of immigration on extinction. *Ecology*, *58*, 445–449. https://doi.org/10.2307/1935620

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). New York, NY: Springer-Verlag.

Caltrans. (2017). *State Highway Network (SHN) and Postmile system*. https://gisdatacaltrans.opendata.arcgis.com/datasets/e21659aeca804a97b7e5265ffa76351c_0

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, *22*, 3124–3140. https://doi.org/10.1111/mec.12354

Clarke, R. T., Rothery, P., & Raybould, A. F. (2002). Confidence limits for regression relationships between distance matrices: Estimating gene flow with distance. *Journal of Agricultural, Biological, and Environmental Statistics*, *7*, 361–372. https://doi.org/10.1198/108571102320

Crooks, K. R. (2002). Relative sensitivites of mammalian carnivores to habitat fragmentation. *Conservation Biology*, *16*, 488–502.

Crooks, K. R., Riley, S. P. D., Gehrt, S. D., Gosselink, T. E., & van Deelen, T. R. (2010). Community ecology of urban carnivores. In S. D. Gehrt, S. P. D. Riley, & B. L. Cypher (Eds.), *Urban carnivores: Ecology, conflict, and conservation* (pp. 185–198). Baltimore, MD: The Johns Hopkins University Press.

Crooks, K. R., & Soulé, M. E. (1999). Mesopredator release and avifaunal extinctions in a fragmented system. *Nature*, *400*, 563–566. https://doi.org/10.1038/23028

Cushman, S. A., & Landguth, E. L. (2010a). Scale dependent inference in landscape genetics. *Landscape Ecology*, *25*, 967–979. https://doi.org/10.1007/s10980-010-9467-0

Cushman, S. A., & Landguth, E. L. (2010b). Spurious correlations and inference in landscape genetics. *Molecular Ecology*, *19*, 3592–3602. https://doi.org/10.1111/j.1365-294X.2010.04656.x

Dixon, J. D., Oli, M. K., Wooten, M. C., Eason, T. H., McCown, J. W., & Cunningham, M. W. (2007). Genetic consequences of habitat fragmentation and loss: The case of the Florida black bear (*Ursus americanus floridanus*). *Conservation Genetics*, *8*, 455–464. https://doi.org/10.1007/s10592-006-9184-z

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NEESTIMATOR v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*, *14*, 209–214.

Dobson, A. P., Rodriguez, J. P., Roberts, W. M., & Wilcove, D. S. (1997). Geographic distribution of endangered species in the United States. *Science*, *275*, 550–553. https://doi.org/10.1126/science.275.5299.550

Ernest, H. B., Vickers, T. W., Morrison, S. A., Buchalski, M. R., & Boyce, W. M. (2014). Fractured genetic connectivity threatens a Southern California puma (*Puma concolor*) population. *PLoS ONE*, *9*, e107985. https://doi.org/10.1371/journal.pone.0107985

Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., … Wardle, D. A. (2011). Trophic downgrading of planet Earth. *Science*, *333*, 301–306. https://doi.org/10.1126/science.1205106

Evans, J. S., Oakleaf, J., Cushman, S. A., & Theobald, D. (2014). *An* ARCGIS *toolbox for surface gradient and geomorphometric modeling, version 2.0-0*. Laramie, WY: The Nature Conservancy and University of Wyoming. https://evansmurphy.wixsite.com/evansspatial

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 487–515. https://doi.org/10.1146/annurev.ecolsys.34.011802.132419

Fischer, M. C., Rellstab, C., Leuzinger, M., Roumet, M., Gugerli, F., Shimizu, K. K., … Widmer, A. (2017). Estimating genomic diversity and population differentiation - an empirical comparison of microsatellite and SNP variation in Arabidopsis halleri. *BMC Genomics*, *18*, 1–15. https://doi.org/10.1186/s12864-016-3459-7

Fountain-Jones, N. M., Craft, M. E., Funk, W. C., Kozakiewicz, C., Trumbo, D. R., Boydston, E. E., … Carver, S. (2017). Urban landscapes can change virus gene flow and evolution in a fragmentation-sensitive carnivore. *Molecular Ecology*, *26*, 6487–6498. https://doi.org/10.1111/mec.14375

Fuller, T. K., DeStefano, S., & Warren, P. S. (2010). Carnivore behavior and ecology, and relationship to urbanization. In S. D. Gehrt, S. P. D. Riley, & B. L. Cypher (Eds.), *Urban carnivores: Ecology, conflict, and conservation* (pp. 13–19). Baltimore, MD: The Johns Hopkins University Press.

Funk, W. C., Blouin, M. S., Corn, P. S., Maxell, B. A., Pilliod, D. S., Amish, S., & Allendorf, F. W. (2005). Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, *14*, 483–496. https://doi.org/10.1111/j.1365-294X.2005.02426.x

Goldberg, C. S., & Waits, L. P. (2010). Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. *Molecular Ecology*, *19*, 3650–3663. https://doi.org/10.1111/j.1365-294X.2010.04673.x

Helyar, S. J., Hemmer-hansen, J., Bekkevold, D., Taylor, M. I., Ogden, R., Limborg, M. T., … Nielsen, E. E. (2011). Application of SNPs for population genetics of nonmodel organisms: New opportunities and challenges. *Molecular Ecology Resources*, *11*, 123–136. https://doi.org/10.1111/j.1755-0998.2010.02943.x

Hilty, J. A., & Merenlender, A. M. (2004). Use of riparian corridors and vineyards by mammalian predators in northern California. *Conservation Biology*, *18*, 126–135. https://doi.org/10.1111/j.1523-1739.2004.00225.x

Hoffmann, A. A., Sgrò, C. M., & Kristensen, T. N. (2017). Revisiting adaptive potential, population size, and conservation. *Trends in Ecology and Evolution*, *32*, 506–517. https://doi.org/10.1016/j.tree.2017.03.012

Holderegger, R., & Wagner, H. H. (2008). Landscape genetics. *BioScience*, *58*, 199–207. https://doi.org/10.1641/B580306

Homer, C., Dewitz, J., Yang, L., Jin, S., Danielson, P., Xian, G., … Megown, K. (2015). Completion of the 2011 National Land Cover Database for the conterminous United States – representing a decade of land cover change information. *Photogrammetric Engineering & Remote Sensing*, *81*, 345–354.

Hunter, R. D., Fisher, R. N., & Crooks, K. R. (2003). Landscape-level connectivity in coastal southern California, USA, as assessed through carnivore habitat suitability. *Natural Areas Journal*, *23*, 302–314. Retrieved from http://pubs.er.usgs.gov/publication/70025580

Jennings, M., & Lewison, R. (2013). *Planning for connectivity under climate change: Using bobcat movement to assess landscape connectivity across San Diego County's open spaces*. Report for the San Diego Foundation, Blasker Final Report, 50 pp.

Jennings, M., & Zeller, K. (2017). *Comprehensive multi-species connectivity assessment and planning for the highway 67 region of San Diego County*. Final Report for the San Diego Association of Governments, 77 pp.

Johnson, M. T. J., & Munshi-South, J. (2017). Evolution of life in urban environments. *Science*, 358. https://doi.org/10.1126/science.aam8327

Jombart, T. (2008). ADEGENET: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Kalinowski, S. T. (2005). HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, *5*, 187–189. https://doi.org/10.1111/j.1471-8286.2004.00845.x

Kamvar, Z. N., Brooks, J. C., & Grünwald, N. J. (2015). Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics*, *6*, 1–10. https://doi.org/10.3389/fgene.2015.00208

Keenan, K., Mcginnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). DIVERSITY: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, *4*, 782–788.

Keyghobadi, N. (2007). The genetic implications of habitat fragmentation for animals. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, *85*, 1049–1064.

Kozakiewicz, C. P., Burridge, C. P., Funk, W. C., Salerno, P. E., Trumbo, D. R., Gagne, R. B., … Carver, S. (2019). Data from: Urbanization reduces genetic connectivity in bobcats (*Lynx rufus*) at both intra- and interpopulation spatial scales. Dryad Digital Repository. https://doi.org/10.5061/dryad.8pk0p2nhq

LaPoint, S., Balkenhol, N., Hale, J., Sadler, J., & van der Ree, R. (2015). Ecological connectivity research in urban areas. *Functional Ecology*, *29*, 868–878. https://doi.org/10.1111/1365-2435.12489

Lee, J. S., Ruell, E. W., Boydston, E. E., Lyren, L. M., Alonso, R. S., Troyer, J. L., … Vandewoude, S. (2012). Gene flow and pathogen transmission among bobcats (*Lynx rufus*) in a fragmented urban landscape. *Molecular Ecology*, *21*, 1617–1631.

Lu, Z., Johnson, W. E., Menotti-Raymond, M., Yuhki, N., Martenson, J. S., Mainka, S., … O'Brien, S. J. (2001). Patterns of genetic diversity in remaining giant panda populations. *Conservation Biology*, *15*, 1596–1607. https://doi.org/10.1046/j.1523-1739.2001.00086.x

Luu, K., Bazin, E., & Blum, M. G. B. (2017). PCADAPT: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, *17*, 67–77.

Lyren, L. M., Alonso, R. S., Crooks, K. R., & Boydston, E. E. (2008a). *Evaluation of functional connectivity for bobcats and coyotes across the former El Toro Marine Base, Orange County, California*. Administrative Report, 179.

Lyren, L. M., Alonso, R. S., Crooks, K. R., & Boydston, E. E. (2008b). *GPS telemetry, camera trap, and mortality surveys of bobcats in the San Joaquin Hills, Orange County, California*. Administrative Report, 134 pp.

Lyren, L. M., Turschak, G. M., Ambat, E. S., Haas, C. D., Tracey, J. A., Boydston, E. E., … Crooks, K. R. (2006). *Carnivore activity and movement in a Southern California protected area, the North/Central Irvine Ranch*. U.S. Geological Survey Technical Report, 115 pp.

Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, *18*, 189–197. https://doi.org/10.1016/S0169-5347(03)00008-9

Markovchick-Nicholls, L., Regan, H. M., Deutschman, D. H., Widyanata, A., Martin, B., Noreke, L., & Ann Hunt, T. (2008). Relationships between human disturbance and wildlife land use in urban habitat fragments. *Conservation Biology*, *22*, 99–109. https://doi.org/10.1111/j.1523-1739.2007.00846.x

Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piñero, D., & Emerson, B. C. (2015). Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. *Molecular Ecology Resources*, *15*, 28–41.

McRae, B. H., Dickson, B. G., Keitt, T. H., & Shah, V. B. (2008). Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology*, *89*, 2712–2724. https://doi.org/10.1890/07-1861.1

Miles, L. S., Dyer, R. J., & Verrelli, B. C. (2018). Urban hubs of connectivity: Contrasting patterns of gene flow within and among cities in the western black widow spider. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20181224.

Murcia, C. (1995). Edge effects in fragmented forests: Implications for conservation. *Trends in Ecology & Evolution*, *10*, 58–62. https://doi.org/10.1016/S0169-5347(00)88977-6

Murphy, M. A., Evans, J. S., & Storfer, A. (2010). Quantifying *Bufo boreas* connectivity in Yellowstone National Park with landscape genetics. *Ecology*, *91*, 252–261. https://doi.org/10.1890/08-0879.1

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853–858. https://doi.org/10.1038/35002501

Noss, R. F., Quigley, H. B., Hornocker, M. G., Merrill, T., & Paquet, P. C. (1996). Conservation biology and carnivore conservation in the Rocky Mountains. *Conservation Biology*, *10*, 949–963. https://doi.org/10.1046/j.1523-1739.1996.10040949.x

Ordeñana, M. A., Crooks, K. R., Boydston, E. E., Fisher, R. N., Lyren, L. M., Siudyla, S., … Van Vuren, D. H. (2010). Effects of urbanization on carnivore species distribution and richness. *Journal of Mammalogy*, *91*, 1322–1331. https://doi.org/10.1644/09-MAMM-A-312.1

Paris, J. R., Stevens, J. R., & Catchen, J. M. (2017). Lost in parameter space: A road map for Stacks. *Methods in Ecology and Evolution*, *8*, 1360–1373.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, *7*, e37135. https://doi.org/10.1371/journal.pone.0037135

Poessel, S. A., Burdett, C. L., Boydston, E. E., Lyren, L. M., Alonso, R. S., Fisher, R. N., & Crooks, K. R. (2014). Roads influence movement and home ranges of a fragmentation-sensitive carnivore, the bobcat, in an urban landscape. *Biological Conservation*, *180*, 224–232. https://doi.org/10.1016/j.biocon.2014.10.010

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, *81*, 559–575. https://doi.org/10.1086/519795

R Development Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raj, A., Stephens, M., & Pritchard, J. K. (2014). FASTSTRUCTURE: Variational inference of population structure in large SNP data sets. *Genetics*, *197*, 573–589. https://doi.org/10.1534/genetics.114.164350

Ramalho, C. E., & Hobbs, R. J. (2012). Time for a change: Dynamic urban ecology. *Trends in Ecology and Evolution*, *27*, 179–188. https://doi.org/10.1016/j.tree.2011.10.008

Randa, L. A., & Yunger, J. A. (2006). Carnivore occurrence along an urban–rural gradient: A landscape-level analysis. *Journal of Mammalogy*, *87*, 1154–1164. https://doi.org/10.1644/05-MAMM-A-224R2.1

Reding, D. M., Cushman, S. A., Gosselink, T. E., & Clark, W. R. (2013). Linking movement behavior and fine-scale genetic structure to model landscape connectivity for bobcats (*Lynx rufus*). *Landscape Ecology*, *28*, 471–486. https://doi.org/10.1007/s10980-012-9844-y

Riley, S. P. D. (2006). Spatial ecology of bobcats and gray foxes in urban and rural zones of a national park. *The Journal of Wildlife Management*, *70*, 1425–1435. https://doi.org/10.2193/0022-541X(2006)70[1425:SEOBAG]2.0.CO;2

Riley, S. P. D., Boydston, E. E., Crooks, K. R., & Lyren, L. M. (2010). Bobcats (*Lynx rufus*). In S. D. Gehrt, S. P. D. Riley, & B. L. Cypher (Eds.), *Urban carnivores: Ecology, conflict, and conservation* (pp. 121–138). Baltimore, MD: The Johns Hopkins University Press.

Riley, S. P. D., Pollinger, J. P., Sauvajot, R. M., York, E. C., Bromley, C., Fuller, T. K., & Wayne, R. K. (2006). A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology*, *15*, 1733–1741. https://doi.org/10.1111/j.1365-294X.2006.02907.x

Riley, S. P. D., Sauvajot, R. M., Fuller, T. K., York, E. C., Kamradt, D. A., Bromley, C., & Wayne, R. K. (2003). Effects of urbanization and habitat fragmentation on bobcats and coyotes in southern California. *Conservation Biology*, *17*, 566–576. https://doi.org/10.1046/j.1523-1739.2003.01458.x

Riley, S. P. D., Serieys, L. E. K., Pollinger, J. P., Sikich, J. A., Dalbeck, L., Wayne, R. K., & Ernest, H. B. (2014). Individual behaviors dominate the dynamics of an urban mountain lion population isolated by roads. *Current Biology*, *24*, 1989–1994. https://doi.org/10.1016/j.cub.2014.07.029

Rivkin, L. R., Santangelo, J. S., Alberti, M., Aronson, M. F. J., de Keyzer, C. W., Diamond, S. E., … Johnson, M. T. J. (2019). A roadmap for urban evolutionary ecology. *Evolutionary Applications*, *12*, 384–398. https://doi.org/10.1111/eva.12734

Robertson, J. M., Murphy, M. A., Pearl, C. A., Adams, M. J., Páez-Vacas, M. I., Haig, S. M., … Funk, W. C. (2018). Regional variation in drivers of connectivity for two frog species (*Rana pretiosa* and *R. luteiventris*) from the U.S. *Pacific Northwest*. *Molecular Ecology*, *27*, 3242–3256.

Roelke, M. E., Martenson, J. S., & O'Brien, S. J. (1993). The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology*, *3*, 340–350. https://doi.org/10.1016/0960-9822(93)90197-V

Row, J. R., Doherty, K. E., Cross, T. B., Schwartz, M. K., Oyler-McCance, S. J., Naugle, D. E., … Fedy, B. C. (2018). Quantifying functional connectivity: The role of breeding habitat, abundance, and landscape features on range-wide gene flow in sage-grouse. *Evolutionary Applications*, 1–17. https://doi.org/10.1111/eva.12627

Row, J. R., Knick, S. T., Oyler-McCance, S. J., Lougheed, S. C., & Fedy, B. C. (2017). Developing approaches for linear mixed modeling in landscape genetics through landscape-directed dispersal simulations.

Ecology and Evolution, *7*, 3751–3761. https://doi.org/10.1002/ece3.2825

Ruell, E. W., Riley, S., Douglas, M. R., Antolin, M. F., Pollinger, J. R., Tracey, J. A., … Crooks, K. R. (2012). Urban habitat fragmentation and genetic population structure of bobcats in coastal southern California. *The American Midland Naturalist*, *168*, 265–280. https://doi.org/10.1674/0003-0031-168.2.265

Santure, A. W., Stapley, J., Ball, A. D., Birkhead, T. R., Burke, T., & Slate, J. (2010). On the use of large marker panels to estimate inbreeding and relatedness: Empirical and simulation studies of a pedigreed zebra finch population typed at 771 SNPs. *Molecular Ecology*, *19*, 1439–1451. https://doi.org/10.1111/j.1365-294X.2010.04554.x

Serieys, L. E., Lea, A., Pollinger, J. P., Riley, S. P., & Wayne, R. K. (2015). Disease and freeways drive genetic change in urban bobcat populations. *Evolutionary Applications*, *8*, 75–92. https://doi.org/10.1111/eva.12226

Shirk, A. J., Landguth, E. L., & Cushman, S. A. (2018). A comparison of regression methods for model selection in individual-based landscape genetic analysis. *Molecular Ecology Resources*, *18*, 55–67. https://doi.org/10.1111/1755-0998.12709

Short bull, R. A., Cushman, S. A., Mace, R., Chilton, T., Kendall, K. C., Landguth, E. L., … Luikart, G. (2011). Why replication is important in landscape genetics: American black bear in the Rocky Mountains. *Molecular Ecology*, *20*, 1092–1107. https://doi.org/10.1111/j.1365-294X.2010.04944.x

Taylor, P. D., Fahrig, L., Henein, K., & Merriam, G. (1993). Connectivity is a vital element of landscape structure. *Oikos*, *68*, 571–573. https://doi.org/10.2307/3544927

Thomassen, H. A., Harrigan, R. J., Semple Delaney, K., Riley, S. P. D., Serieys, L. E. K., Pease, K., … Smith, T. B. (2018). Determining the drivers of population structure in a highly urbanized landscape to inform conservation planning. *Conservation Biology*, *32*, 148–158. https://doi.org/10.1111/cobi.12969

Tigas, L. A., Van Vuren, D. H., & Sauvajot, R. M. (2002). Behavioral responses of bobcats and coyotes to habitat fragmentation and corridors in an urban environment. *Biological Conservation*, *108*, 299–306. https://doi.org/10.1016/S0006-3207(02)00120-9

Tracey, J. A., Zhu, J., Boydston, E., Lyren, L., Fisher, R. N., & Crooks, K. R. (2013). Mapping behavioral landscapes for animal movement: A finite mixture modeling approach. *Ecological Applications*, *23*, 654–669. https://doi.org/10.1890/12-0687.1

Tucker, S. A., Clark, W. R., & Gosselink, T. E. (2008). Space use and habitat selection by bobcats in the fragmented landscape of south-central Iowa. *Journal of Wildlife Management*, *72*, 1114–1124. https://doi.org/10.2193/2007-291

U. S. Census Bureau (2010). 2010 Census of Population and Housing. *Population and Housing Unit Counts*. Available from https://www.census.gov/library/publications/2012/dec/cph-2.html

U. S. Census Bureau (2016). *American Community Survey 1-year estimates*. Available from https://www.census.gov/newsroom/press-kits/2017/acs-one-year.html

Van Strien, M. J., Keller, D., & Holderegger, R. (2012). A new analytical approach to landscape genetic modelling: Least-cost transect analysis and linear mixed models. *Molecular Ecology*, *21*, 4010–4023. https://doi.org/10.1111/j.1365-294X.2012.05687.x

Vandergast, A. G., Bohonak, A. J., Hathaway, S. A., Boys, J., & Fisher, R. N. (2008). Are hotspots of evolutionary potential adequately protected in southern California? *Biological Conservation*, *141*, 1648–1664. https://doi.org/10.1016/j.biocon.2008.04.009

Vandergast, A. G., Bohonak, A. J., Weissman, D. B., & Fisher, R. N. (2007). Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: Phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: Stenopelmatus). *Molecular Ecology*, *16*, 977–992. https://doi.org/10.1111/j.1365-294X.2006.03216.x

Wang, I. J. (2009). Fine-scale population structure in a desert amphibian: Landscape genetics of the black toad (*Bufo exsul*). *Molecular Ecology*, *18*, 3847–3856.

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, *23*, 5649–5662. https://doi.org/10.1111/mec.12938

Waples, R. S., & England, P. R. (2011). Estimating contemporary effective population size on the basis of linkage disequilibrium in the face of migration. *Genetics*, *189*, 633–644. https://doi.org/10.1534/genetics.111.132233

Waples, R. K., Larson, W. A., & Waples, R. S. (2016). Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity*, *117*, 233–240. https://doi.org/10.1038/hdy.2016.60

Wickham, H., & Chang, W. (2008). ggplot2: An implementation of the Grammar of Graphics. R package version 3.2.1. Available from https://cran.r-project.org/web/packages/ggplot2/index.html

Wright, S. (1943). Isolation by distance. *Genetics*, *28*, 114–138.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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